

THE MOUTH PARTS, ALIMENTARY TRACT, AND SALIVARY APPARATUS OF THE FEMALE IN *PHLEBOTOMUS PAPATASII*

BY

S. ADLER

AND

O. THEODOR

(MICROBIOLOGICAL INSTITUTE, JERUSALEM)

(Received for publication 27 November, 1925)

PLATES VIII-XIV

The external morphology of the mouth parts of *Phlebotomus papatasi* has been described by Grassi (1907) and by Newstead (1911), but so far as we are aware, a detailed study of the biting, pumping and salivary apparatus has not been made for any member of the genus *Phlebotomus*.

The following study was made on *Phlebotomus papatasi*, the commonest sandfly of Palestine. The method of study adopted was observation of the mouth parts *in vivo*, dissection and serial sections, transverse, sagittal and coronal of the head.

Observation *in vivo* is the most satisfactory method of studying the action of the mouth parts. A sandfly is lightly anaesthetised with ether so that it remains motionless for about ten minutes, during which time the mouth parts are usually working actively, so that their movements can be observed under the microscope, and the contractions of the muscles of the buccal cavity can also be seen and counted. If the insects are stunned by shaking them vigorously in a test-tube, and their wings and legs removed, the movements of the external mouth parts and the contraction of the muscles of the buccal cavity will often continue up to four hours, during which period they can be conveniently studied.

Dissection of the mouth parts can be made in freshly-killed insects, but for permanent mounted preparations it is advisable to leave the insects overnight in a 5 per cent. solution of potash before dissecting.

Serial sections were made after fixation in Henning's solution or Dobell's fluid, as recommended by Hoare (1921).

There are several formulae for Henning's solution differing in the amount of nitric acid they contain; the following formula, taken from Bolles Lee (1921), was found useful for sandflies and mosquitos.

Nitric acid	16 parts
Chromic acid, 0.5 per cent.	16 parts
Saturated solution of mercuric chloride in 60 per cent. alcohol	24 parts
Saturated solution of picric acid in water	12 parts
Absolute alcohol	42 parts

After fixation in Henning's solution for one day, the insects were placed for two hours in a mixture of equal parts Lugol's solution and 70 per cent. alcohol; they were then passed through changes of 70 per cent. alcohol till no trace of iodine remained. Some specimens were passed through alcohol and paraffin in the usual way and others were stained in bulk, either in a saturated solution of eosin in 70 per cent. alcohol for two days, or in haematoxylin for three days, and then treated in the usual manner. After fixation in Henning's solution for twenty-four hours, sections cannot be cut less than 20μ thick. For a study of the musculature of the mouth parts and head, sections 50μ thick are the most convenient. Staining in bulk for three days in Ehrlich's haematoxylin gives the best results for histological purposes after fixation in Henning's solution.

Dobell's process is very satisfactory for histological purposes, for after fixation in Dobell's fluid, sections 7μ thick can be obtained, but the process is a lengthy one and is not necessary for the study of the muscular system.

MOUTH PARTS

The proboscis consists of the labrum-epipharynx, hypopharynx, two mandibles, two maxillae and a labium. The length of the proboscis from the mouth to the tip of the epipharynx is 400μ . The armed parts when at rest lie unsheathed in the labium in the following manner: the labrum-epipharynx is superior and the two mandibles lie one above the other immediately between the epipharynx and hypopharynx; the maxillae lie beneath and lateral

to the hypopharynx and for the greater part of their course appear moulded to the inferior lateral aspect of the latter. It will be seen from Pl. IX, figs. 7 and 8, Pl. XIII, figs. 14 and 15, that when the parts are at rest the epipharynx and one of the mandibles form a canal, the roof of which is a groove on the inferior surface of the epipharynx, and the floor the upper surface of one of the mandibles. This canal is directly continuous with the buccal cavity and we have found it to contain *Herpetomonas* five days after feeding on an oriental sore.

The distal ends of the epipharynx, hypopharynx and mandibles lie at the extremity of the labium between the labella, and the distal ends of the maxillae lie a varying distance behind them. Owing to the disposition of the mandibles the teeth of the epipharynx and hypopharynx are never in contact when the insect is at rest.

The labrum-epipharynx is 400μ long. The labrum is a thin chitinous band which rises from the anterior superior end of the clypeus and is attached to the epipharynx first by a narrow strip of muscle and then by loose membranous tissue; it becomes fused to the epipharynx a little behind the distal end of the latter.

The epipharynx is 400μ long and 40μ at its broadest part; the middle of its lower surface is grooved by a channel which, in transverse section, is triangular, the apex of the triangle being rounded (Pl. IX, figs. 7 and 8, Pl. XIII, figs. 13-15); this channel forms the roof of the food canal during the act of feeding. The distal end of the epipharynx is pointed and toothed, each tooth being curved and pointing forwards, and its toothed margin is concave.

The hypopharynx is 400μ long and 40μ at its broadest part. It is pierced through the whole of its length by the salivary canal; its distal end is toothed, the teeth being smaller than those of the epipharynx, and the toothed margin is convex. The upper surface of the hypopharynx forms the floor of the food canal during the act of feeding.

The mandibles are 420μ long and 30μ in their broadest part, and rise from the clypeus above, behind, and lateral to the mouth. The base of the mandible is divided into two cornua, one external and one internal. Rising from the infero-lateral aspect of the clypeus is a sclerite (Pl. XI, fig. 2) which curves upwards and inwards and terminates in a free point at the side of the clypeus; the free end of this sclerite lies between the two cornua of the mandible (Pl. XI,

fig. 2) and plays an important part in regulating the movements of the latter. The cornua of the mandible are darkly pigmented and form a well-marked external feature of the cranium of *Phlebotomus papatasi*.

The mandible passes downwards and inwards for a short distance and the inner side of this portion shows a strongly-marked ridge into which the adductor muscle of the mandible is inserted. Beyond the adductor ridge the mandible turns inwards and passes straight downwards between the epipharynx and hypopharynx. The distal end of the mandible is sharply pointed and its inner margin is serrated for a distance of 60μ (Pl. XI, fig. 1). The tip of each mandible is seen at the opposite side of the proboscis distally (Pl. XIV, fig. 2).

The maxillae are composed of two parts, a blade which is extra-cranial and a long process which is intra-cranial. The blade is 330μ long and 30μ in its broadest part; at its distal end, externally, there are five or six tooth-like processes pointing backwards and gradually diminishing in size from before backwards; a part of the internal margin, 120μ long and commencing 35μ behind the distal end, is also armed with tooth-like processes which point forwards. The long process of the maxillae (Pl. XI, fig. 3) is a rod of chitin 325μ long which runs backwards along the floor of the cranium; in a cleared preparation of the head it appears lateral and inferior to the whole of the buccal cavity and a part of the pharynx; it is attached by a broad chitinous band to the floor of the cranium and the points of attachment on the two sides are connected by a thin strip of membrane (Pl. XI, fig. 3).

The labium, when at rest, is 420μ long and 110μ at its broadest part, i.e., at the level of the labella, and is, with the exception of several structures to be described later, a soft organ with membranous walls. The ventral and lateral surfaces form the mentum and the concave dorsal surface forms the labial gutter (Pl. IX, figs. 7 and 8), in which the armed parts are ensheathed when the insect is at rest.

The mentum rises from a horse-shoe shaped piece of chitin (Pl. XIV, figs. 3 and 4) which forms the anterior end of the gular region and serves as the origin of the intrinsic labial muscles. The labium itself contains, in addition to the muscles, a large amount of fat, two main tracheae and their branches and the two labial nerves which rise from the middle of the lower border of the inferior ganglion

of the brain. The cavity of the labium is connected by a wide opening with the body cavity. The two labella are articulated by a chitinous disc to the labium. A median lobe or glossa, according to Grassi's terminology, lies between the two labella. The structure of the labella and glossa can only be suitably studied in fresh preparations when the whole labium is contracting and the labella become separated and the glossa distended.

When the labium is at rest the median ends of the chitinous discs which form the base of the labella are almost in contact (Pl. XIV, fig. 3), the glossa is almost completely hidden from view and its details have therefore been overlooked in previous descriptions.

On the ventral side the median end of the base of the labellum is articulated through a small rod of chitin (Pl. XIV, fig. 1) to an elongated chitinous rod which passes inwards and backwards to unite with its fellow from the other side (Pl. XIV, figs. 1, 3 and 4), and form a support for the ventral side of the labium.

On the median half of the dorsal surface of the labella are a number of fine, closely-set lines or grooves running almost horizontally (Pl. XIV, fig. 1) which produce the appearance of a pseudo-tracheal membrane. Near the distal ends of each labellum, dorsally, are four pre-stomal teeth, three median, which are close together, and one individual tooth external and more distal than the others (Pl. XIV, fig. 1). A minute muscle is inserted into the base of each tooth.

Inside each labellum near the distal end there is a group of nerve cells.

Ventrally each labellum is divided into a proximal and a distal half by a fine chitinous line (Pl. XIV, fig. 1) which, after pursuing a horizontal course till it almost reaches the internal border of the labellum, passes backwards to join the median end of the base of the latter. On the distal half of the labellum, ventrally, are a number of long and rather stout hairs.

The glossa (Pl. XIV, figs. 1, 2 and 4) is a transparent structure divided into two by a median longitudinal line; running through the greater part of each half is a thick chitinous line which is attached proximally to the median end of the base of the labella (Pl. XIV, fig. 1); from this chitinous line others radiate and the glossa folds up along these chitinous lines when the labium returns to the resting position. Distally the dorsal surface of the glossa is finely corrugated.

The palps have been amply described by Newstead, who noted that the fourth and fifth segments are bent downwards and backwards in such a way that the palps protect the proboscis. The third segment extends slightly in front of the proboscis. In Palestine *P. papatasii* shows an interesting local variation from the same species as described by Newstead, from Malta. In Malta, Newstead found that the fourth and fifth segments are distinctly annulated; in Palestine the second and third segments are also distinctly annulated.

THE MUSCULATURE OF THE MOUTH PARTS AND THE METHOD OF BITING

In anaesthetised specimens of freshly-killed sandflies it is seen that the greater part of the gular region moves backwards and forwards in one plane. The part of the gular region that is involved in this movement is separated by a fold of membrane from a narrow area which extends in front of the occipital foramen and is bounded latero-posteriorly by the narrow inferior openings of the two intra-cranial tunnels.

During retraction the fold between the motile part of the gular region and the narrow area in front of the occipital foramen deepens and the membranous floor of the motile portion bulges downwards.

The intra-cranial tunnels are two hollow chitinous rods (Pl. XI, fig. 4), one on each side, which are connected with the exterior, superiorly and anteriorly, by a wide funnel-shaped opening in front of the base of the first antennal segment, and inferiorly and posteriorly by a narrow opening at the level of the posterior margin of the eye. Anteriorly they are connected by transverse chitinous tubes which unite in the middle line and are produced backwards as a chitinous bar which, after a short distance, branches into two chitinous bands which fuse with the roof of the cranium (Pl. XI, fig. 4). The superior ends of the intra-cranial tunnels serve as an origin for the intra-cranial muscles to the first antennal segment.

The muscles responsible for this movement are: (1) a pair of powerful protractor muscles (Pl. XII, fig. 1) which rise, one on each side, from the roof of the clypeus and passing downwards and backwards are inserted into the posterior part of the movable

portion of the gular region ; (2) a pair of retractor muscles, one on each side, which rise partly from the floor of the cranium immediately behind the inferior opening of the intra-cranial tunnel and partly from the lower part of the intra-cranial tunnel, and are inserted into the extreme anterior end of the gular region (Pl. XII, fig. 1).

The labium, maxillae and palps are attached to the gular region and they are, therefore, carried backwards and forwards during the similar movements of the latter.

The maxillae and palps have, in addition, muscles peculiar to themselves. The maxilla is supplied by a muscle which rises from the roof of the clypeus behind the protractor of the gular region and passes downwards and forwards to be inserted into the long process of the maxilla a little behind the junction of the latter with the blade (Pl. XII, fig. 2) ; thus although receiving directly only the insertion of one muscle, the maxilla is acted upon by three muscles, owing to its attachment to the floor of the cranium, and of all the armed mouth parts the maxilla has directly and indirectly the largest and most powerful supply of muscles.

From the posterior part of the floor of the movable portion of the gular region a muscle arises which passes forwards and slightly upwards to be inserted into the first segment of the palps and acts as an elevator and abductor of the palps. During the act of feeding the palps are elevated and abducted.

The mandibles are supplied by two relatively powerful muscles (Pl. XII, fig. 2) which rise from the posterior part of the cranium laterally and inferiorly and pass forwards and slightly upwards. The internal and narrower of these two muscles (Pl. XII, fig. 2, mm₁) is inserted into the adductor ridge of the mandible, and the external and broader muscle (Pl. XII, fig. 2) is inserted into the tip of the external cornu. The external muscle, shortly before its insertion into the external cornu, turns round an invagination into the side of the clypeus and passes outwards for a short distance ; this muscle abducts and rotates the mandible externally. The internal muscle pursues a straight course forwards and medianwards towards insertion and, on contraction, causes adduction and internal rotation of the mandible. When the mouth parts are in action the mandibles are rapidly abducted and adducted through a narrow angle and are at

the same time rotated, movements which can be readily followed *in vivo* under the microscope. Abduction and adduction are both limited by the wedge of chitin which lies between the two cornua, for when the mandible is abducted through a narrow angle the external cornu is pressed against the wedge of chitin and further abduction is impossible; when the mandible is adducted through a narrow angle the internal cornu is pressed against the wedge of chitin and further adduction is impossible. When the mandibles are in action it is seen that the cornua move in an arc round and in front of the wedge of chitin lying between them; the mandibles, as judged by the movements of the cornua, also undergo a limited amount of backward and forward movements owing to the elasticity of the sclerite from which they rise. During the act of biting the inner dorsal margin of the expanded labella also imposes a limit to the abduction of the two mandibles.

The epipharynx and labrum are supplied by laterally symmetrical muscles, two of which function as regulators of the diameter of the food canal during the act of feeding (Pl. XII, fig. 1).

These muscles are: (1) a muscle which arises from the commencement of the labrum and, passing downwards and slightly forwards, is inserted into the epipharynx; (2) a muscle which arises from the roof of the clypeus posteriorly and, passing obliquely downwards and forwards, is inserted into the junction of the epipharynx and roof of the buccal cavity.

A third muscle arises from the anterior part of the roof of the clypeus and, passing through the origin of the labrum, is inserted into the under surface of the latter.

We are now in a position to understand the rôles played by the various armed mouth parts in biting. The maxillae, mandibles, epipharynx and hypopharynx all act as piercing stylets, but the most important rôle is played by the maxillae, for they have the widest range of movement backwards and forwards, and directly and indirectly possess the most powerful muscle supply of all the biting parts. The mandibles, through their rapid movements of adduction, abduction and rotation, combined with the small backward and forward movements described above, penetrate and enlarge the wound in all directions.

Patton and Cragg (1913) are of the opinion that the epipharynx

and hypopharynx do not play the part of active piercing stylets in the mosquito during the act of biting, but it is doubtful whether this view holds in the case of *Phlebotomus papatasi*. We have observed two males of *P. papatasi* containing blood, one of them gorged with fresh blood actually leaving a human being; in both cases the mouth parts were characteristic of the normal male, i.e., the mandibles were absent and the maxillae unarmed. It is interesting to note that in both cases dissection showed the genitalia, external and internal, to be of the normal male type and no trace of hermaphroditism was found.

During the act of feeding the mandibles no longer interpose between the epipharynx and hypopharynx and the two latter coming into opposition form the food canal; their teeth interlock and probably act as a strainer, preventing particles of too large a size from entering the food canal.

The labium undergoes interesting changes during the act of biting. There are two sets of intrinsic longitudinal muscles in the labium, all arising from the chitinous base of the mentum. An external longitudinal muscle is formed by two bellies which arise from the outer part of the base and unite into one tendon which is inserted into the lateral margin of the union of the body of the labium and the base of the labellum (Pl. XIV, fig. 4). Since the labium is a lax structure composed mainly of soft tissues, the external longitudinal muscle causes a decrease of length and an increase of breadth of the whole labium. The internal longitudinal muscle is composed of four bellies which rise from the median part of the base of the labium and pass downwards; at the level of the base of the labella they unite to form a single tendon which passes outwards through the labella and is inserted into the distal end of the latter (Pl. XIV, fig. 4). Contraction of this muscle causes abduction and expansion of the labella. Abduction of the labella is also secured indirectly by the action of the external longitudinal muscle of the labium, for the latter, by increasing the girth of the labium, causes distention and abduction of the labella. As the labella are abducted the median lobe or glossa comes into view; the glossa expands and opens out along the chitinous rays described above in the manner of a fan opening out. When the labium returns to the normal resting position the glossa again folds up. The labium when contracted by the action

of its intrinsic muscles is up to 120μ shorter than the resting labium and it is still further shortened by pressure against the skin; the biting parts projecting beyond the labella have thus ample room for piercing the skin and reaching the blood capillaries.

When the armed mouth parts are in action they lie on the expanded glossa and the median dorsal margin of the labella forms the walls of a groove, guides the mouth parts and restricts the range of their action laterally.

The above description of the movements of the mouth parts of *Phlebotomus papatasi* applies in general principles to the movements of the mouth parts of *P. minutus* and *P. perniciosus* and they may, therefore, be considered as characteristic of the genus *Phlebotomus*.

Phlebotomus papatasi usually bites during the night and early morning but occasionally, under natural conditions, also bites during the day; and in the laboratory specimens which have been starved several days frequently bite and feed readily by day. In our experience about 60 per cent. of specimens under laboratory conditions refuse to feed under any circumstances and die of starvation. Specimens in which the eggs are ripe or nearly ripe usually refuse to feed. *P. papatasi* often bites several times before feeding and we have observed one specimen bite seven times on an area of skin half-an-inch in diameter before feeding. There is an interval of fifteen to thirty seconds between the commencement of the act of biting and the entrance of blood into the buccal cavity; aetiologically this interval is important for it gives an opportunity for parasites in the proboscis to enter the wound. When blood is already flowing into the buccal cavity the negative pressure caused by the muscles of the latter would tend to prevent parasites from the food canal entering the wound.

The buccal cavity is formed by the union of the continuation backwards into the cranium of the epipharynx and hypopharynx; it is composed of three chitinous plates, an inferior one which forms the floor of the cavity and is strongly chitinised, and two lateral plates which meet in the mid-line and are feebly chitinised except at their lateral margins. The continuation of the hypopharynx inside the cranium splits into two laminae, a superior one which is strongly chitinised and convex ventrally, and which forms the floor of the buccal cavity, and an inferior one which is continuous posteriorly

with the inferior part of the common salivary duct (Pl. X, fig. 2). The salivary pump lies between these two laminae.

It is difficult to give an exact verbal description of the shape in transverse section of the buccal cavity, for this differs at various points and can be best appreciated from the figures of transverse sections of the clypeus at various levels (Pl. IX, fig. 6, Pl. XIII, figs. 8-12).

It will be seen that the lumen of the buccal cavity when at rest is very narrow and of a peculiar shape, roughly triangular with the base of the triangle thick and slightly convex downwards, and the sides of the triangle extremely concave inwards.

The lateral part of the backward continuation of the epipharynx is, in marked contrast to the median portion, strongly chitinised, and forms a strong bar of chitin which is fused to the lateral margin of the floor of the buccal cavity. This bar passes upwards and backwards (the general direction of the buccal cavity) and splits into two bars, one inferior and one superior. The inferior bar (Pl. X, figs. 2 and 3) proceeds backwards and then turns downwards and passes below the floor of the buccal cavity, and meeting its fellow from the opposite side forms an arch convex backwards. This arch forms a support for the buccal cavity and during the whole of its course (Pl. XII, fig. 3) serves as an origin for a relatively large and powerful salivary muscle which, converging from all points of the arch, passes downwards and forwards to be inserted into the salivary pump (Pl. VIII, fig. 1, Pl. XII, fig. 3). The upper bar passes upwards and backwards and terminates in a cornu on each side, the two cornua being united by two cross-pieces, one anterior and the other posterior (Pl. X, fig. 3). The connecting tube between the buccal cavity and the pharynx lies below the two cross-pieces.

The buccal cavity is supplied by a large group of muscles which rise from the roof of the clypeus near the middle line and are inserted into the two chitinous plates which form the roof of the buccal cavity. The general direction of these muscles is downwards and forwards, but a few longer than the others pass downwards and backwards from the roof of the clypeus and are inserted into the most posterior part of the buccal cavity (Pl. XII, fig. 4).

The action of the muscles of the buccal cavity can be studied *in vivo*, when they are seen to contract up to one hundred and twenty times a minute; they act as pumping organs, for by creating a

negative pressure they pump blood into the buccal cavity through the food canal. When the muscles contract the two superior chitinous plates are pulled upwards and outwards and the buccal cavity is thus dilated (Pl. XIII, fig. 10).

Immediately behind the buccal cavity and lying below the two cross-pieces is a small chitinous tube 30μ long which joins the buccal cavity to the pharynx. This tube is surrounded by a sphincter muscle which regulates the flow of blood from the buccal cavity into the pharynx (Pl. VIII, fig. 1, Pl. XI, fig. 4),

The pharynx is 210μ long and 63μ in its broadest part; it is broad posteriorly and narrow anteriorly. It is composed of three chitinous plates, one superior and horizontal and two lateral. The transverse section of the lumen of the pharynx varies at different levels but it is roughly triangular in shape, the base of the triangle being superior and all three sides concave internally. Posteriorly ridges are seen on the wall of the pharynx; these are the optical expression of internal teeth which extend for a distance of 80μ along the lateral walls and for a slightly shorter distance along the superior wall. The foremost teeth are small and point backwards and the remainder are vertical (Pl. XII, fig. 4).

The pharynx, except for a small portion posteriorly and another anteriorly, lies inside the brain.

The pharynx is supplied by the following bilaterally symmetrical muscles :

(1) A dorsal anterior group of muscles which rise from the roof of the cranium immediately behind the clypeus and pass downwards to be inserted into the superior plate of the pharynx. The anterior fibres pass vertically downwards and the posterior ones obliquely downwards and backwards between the superior ganglion of the brain and the pharynx (Pl. VIII, fig. 1, Pl. XII, fig. 4).

(2) A dorsal posterior group of muscles which rise from the roof of the cranium above and in front of the occipital foramen and pass downwards to be inserted into the superior plates of the pharynx. The anterior fibres of this group pass obliquely forwards and downwards between the superior ganglion of the brain and the pharynx (Pl. VIII, figs. 1 and 2, Pl. XII, fig. 4).

(3) A powerful group of muscles which rise from the infero-lateral aspect of the cranium posteriorly and pass upwards and forwards to

be inserted into the inferior plates of the pharynx. The anterior fibres of this group pass obliquely upwards and forwards between the pharynx and the inferior ganglion.

Although the greater part of the pharynx lies inside the brain, yet owing to the peculiar direction of the muscles almost the whole surface of the pharynx serves as an insertion for dilator muscles.

The function of the above-described muscles is to pump blood from the buccal cavity into the pharynx, the short tube lying between the pharynx and buccal cavity acting as a regulator of the flow of blood.

The oesophagus is a short tube 80μ long, as measured from the posterior opening of the pharynx to the commencement of the midgut. It is attached for a considerable distance to the sides of the pharynx and thus a pouch is formed between the external wall of the pharynx and the oesophagus (Text-fig. 1). This pouch has been found to contain *Herpetomonas*. The wall of the oesophagus is lined by a single layer of epithelium which lies on a basal membrane; the interior surface of the epithelium is covered with a very fine layer of chitin.

The oesophageal diverticulum lies ventral to the midgut; it opens into the oesophagus at a varying distance from the posterior end of the latter; exceptionally it opens into the pharynx together with the oesophagus. The diverticulum is composed of two very fine layers of muscle fibres, one longitudinal and internal and the other circular and external; internally it is lined with flat epithelium covered by a thin layer of chitin. There is a very narrow sphincter at the junction of the oesophagus and diverticulum. In freshly dissected insects the diverticulum is usually seen to be undergoing peristaltic movements towards the oesophagus. Unlike the oesophageal diverticulum of mosquitos the diverticulum of *P. papatasi* seldom contains air bubbles. Out of four thousand sandflies examined only one instance was observed of an air bubble in the diverticulum and this in spite of the fact that the midgut often contains air bubbles.

Waterston (1922) states that blood can be seen in the oesophageal diverticulum for about forty-eight hours after a meal, while Patton and Cragg (1913) state that the oesophageal diverticulum is filled with blood immediately after a feed but is empty several hours later. In our experience, based on a dissection of four thousand sandflies, it is unusual to find blood or bloodstained fluid in the

oesophageal diverticulum at any time, and in the few cases where blood is found it is present only in negligible quantities as compared with the amount found in the midgut. Even when the insect is fully gorged and the stomach distended to its fullest capacity, the

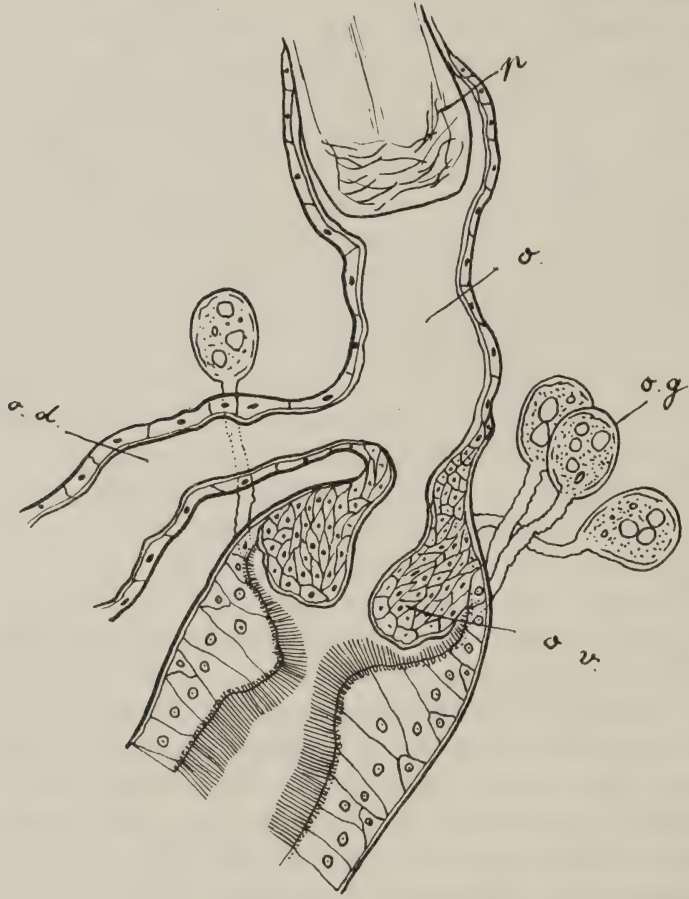


FIG. 1 (diagrammatic). *p.*—pharynx; *o.*—oesophagus; *o.d.*—oesophageal diverticulum; *o.g.*—oesophageal glands; *o.v.*—oesophageal valve.

Note rods projecting from the epithelial cells of the cardia.

Note pouch between the sides of the cardia and the valve.

oesophageal diverticulum contains very few red cells or none at all. Out of twenty-five sandflies killed immediately after a feed four contained red cells in the oesophageal diverticulum, but only in small quantities. In insects killed a few days after a feed

the oesophageal diverticulum is usually found distended with perfectly clear fluid. This fluid is later either absorbed through the thin walls of the diverticulum or is passed into the midgut. In specimens kept after feeding until the midgut is empty, the oesophageal diverticulum is usually also empty. It appears that the oesophageal diverticulum may function as a reservoir, but only for fluids and not for red cells.

There are four small elliptical oesophageal glands 23μ long and 18μ broad, which contain large yellow granules; they lie round the commencement of the midgut and each gland opens by a minute duct into the pouch between the oesophageal valve and the midgut (Text-fig. 1).

The midgut consists of two parts which differ histologically. The upper part or cardia is tubular and lies in the thorax; it is lined by a single layer of columnar epithelium of peculiar structure; near the internal surface of each cell there are a number of fine granules and from each granule a thin transparent rod projects into the lumen of the gut (Text-fig. 1). The rods somewhat resemble cilia but they are not motile or contractile; they are closely packed and in fresh undamaged preparations they are not very evident, but if the wall of the gut is broken by pressure and individual cells set free, their true nature can be readily determined. Each rod is 7.5μ to 10.5μ long. Lying between the epithelial cells near their base there are a number of small interstitial cells.

The oesophageal valve lies at the superior end of the cardia and consists of a downward projection of the oesophagus into the cardia; between this projection and the wall of the midgut there is a small pouch (Text-fig. 1 and Pl. VIII, fig. 2). The posterior surface of the pouch is lined with epithelium characteristic of the cardia.

The structure of the cardia bears an interesting relation to the development of *Herpetomonas* in sandflies. Christophers, Shortt and Barraud (1925) found large numbers of *Herpetomonas* attached to the epithelium of the upper part of the midgut in a number of specimens of *Phlebotomus argentipes* fed on a case of Kala-azar, and the authors have recorded a natural infection of *Phlebotomus papatasi* with *Herpetomonas* in which the parasites were attached to the posterior surface of the oesophageal valve. The authors, in a series of experiments in which one hundred and fifty-five specimens

of *P. papatasi* were fed on oriental sores, found that in nine out of sixteen positive cases flagellates attached themselves to the wall of the cardia, in some cases as early as the third day. The parasites can be seen boring into the epithelium with their flagella, and when they are completely attached their flagella lie entangled among the rods projecting from the epithelial cells and even reach into the cytoplasm. Division of the flagellates takes place mainly in the cardia, particularly in the anterior end which is found in artificial and in some cases in natural infections, to be completely choked up by a mass of flagellates.

The cardia is not distensible, in marked contrast to the second part of the midgut, i.e., the stomach.

The stomach lies in the abdomen and its condition depends on the amount of blood contained and the time from the last feed; it is lined by a single layer of epithelium which does not contain the rods characteristic of the cardia.

The stomach is very distensible and accommodates itself to the relatively enormous feeds of *P. papatasi*. An average female of *P. papatasi* weighs 0.3 milligrams and, after a full feed, weighs 0.4 milligrams. (It is interesting to note that *P. papatasi* never passes blood per rectum during the act of feeding.)

Digestion is a relatively slow process. It is not uncommon to find erythrocytes in a good state of preservation four days after a feed, and in one instance we observed unimpaired erythrocytes eight days after a feed. Haemolysis does not take place as a rule till the third or fourth day after a feed.

A day or two after feeding the distention of the stomach is diminished and the oesophageal diverticulum is filled with a colourless fluid, and during this period no blood is passed in the faeces nor are erythrocytes destroyed in the stomach. Unaltered haemoglobin is never found in the epithelial cells of the stomach but it is passed in the faeces. The above observations tend to show that the essential food element in the blood is the plasma and not the red cells and this is further supported by the fact that sandflies will feed when the red cells from the previous feed are still present in the stomach.

The epithelium of the midgut contains a powerful anticoagulin. In five experiments two small scratches were made close together on

human skin ; the empty midgut of *P. papatasi* was dissected out and rubbed into one scratch and the other was left untreated. Blood oozed from the scratch into which the midgut was rubbed for a considerably longer time, in one instance three-quarters-of-an-hour, than from the other.

The red cells do not come in direct contact with the epithelium of the stomach, for in *P. papatasi* (also in *P. minutus* and *P. perniciosus*) a very definite peritrophic membrane is produced. The peritrophic membrane is readily seen in specimens killed a day or two after a feed ; it is a thin white amorphous structure which contains the mass of red cells. Anteriorly it extends into the cardia and posteriorly into the hindgut. The peritrophic membrane may be likened to a sealed tube closed anteriorly and posteriorly.

The Malpighian tubes rise near the posterior end of the stomach by two common ducts, as described and figured by Newstead (1911). Near their origin the ducts divide into two tubes each about $1,300\mu$ long and 24μ thick ; these extend downwards almost to the end of the abdomen and then curve backwards, their distal extremities lying in the upper part of the abdomen near the thorax.

Immediately behind the origin of the Malpighian tubes there is a ring of muscles which marks off the midgut from the hindgut and serves as a point of origin for the peristaltic movements of the latter. The wall of the hindgut consists of two layers of muscles, one longitudinal and external and the other oblique and internal. The lumen is lined by a single layer of cubical epithelium, the inner aspect of which is covered with an exceedingly thin glistening chitinous layer. In the posterior part of the hindgut are two large rectal papillae 30μ long and 20μ in their widest part ; these are composed of large polygonal cells with a small round nucleus. The rectal papillae are richly supplied with tracheae.

THE SALIVARY APPARATUS

The salivary apparatus consists of the salivary glands, salivary ducts, salivary pump and the salivary channel through the hypopharynx.

The salivary glands lie one on each side in the uppermost ventral part of the thorax. They are hollow, almost spherical organs lined

with a single layer of columnar epithelium which rests on a basal membrane. A fully-distended salivary gland may reach the size of 180μ long by 140μ wide. Immediately after a feed the salivary glands are small and the epithelium thin. If a series of sandflies is dissected at various times after a feed it is seen that the epithelial cells become progressively larger and filled with granules. After a time cells are found free in the lumen of the gland, which contains, in addition, fine granules secreted by the epithelium. The free cells degenerate and break up into refractile granules much larger than those secreted by the epithelium. Three or four days after a feed the gland is distended with secretory granules and the products of degeneration of liberated cells and the epithelium lining the gland is thinned by pressure (Text-fig. 2, *a-e*). It will be seen from the above description that the saliva is composed of the products of liberated cells which degenerate in the lumen and of the secretion of the cells lining the lumen of the gland. Generally there is a parallelism between the condition of the salivary glands and the condition of the stomach.



FIG. 2 (diagrammatic). Salivary glands in various stages. *a*.—immediately after a feed; *b-e*.—subsequent stages.

Note in *c*. free cells in the lumen.

Sandflies which had been kept in the laboratory for four days without food, so that digestion was well advanced and the salivary glands large, were dissected. Twenty salivary glands were placed in 20 cmm. neutral distilled water, the glands were broken up with fine needles and the resulting emulsion transferred to a capillary tube of narrow bore into which a minute amount of phenol red was drawn. The emulsion showed a faint alkaline reaction.

A series of glands were emulsified in 20 cmm. saline and various

amounts of blood were added. The mixtures were drawn into fine capillary tubes and the coagulation time compared with controls containing the same amount of saline and blood. It was found that an emulsion of eight distended salivary glands in 20 cmm. saline delayed coagulation of 2 cmm. human blood for fifteen minutes, and an emulsion of twelve salivary glands delayed the coagulation of 2 cmm. human blood for thirty minutes. (Controls showed complete coagulation in nine minutes.) Since, in nature, the contents of two salivary glands are used for not more than 0.1 cmm. blood, as compared with 0.5 cmm. and 0.3 cmm. in the above experiment, it follows that the saliva functions as an anticoagulant during the act of biting.

Emulsions of ten and twenty salivary glands in 20 cmm. of a 1 per cent. solution of sodium citrate in saline were mixed with 5 cmm. blood and the mixture drawn up in fine capillary tubes and observed under the microscope; no haemolysis and no agglutination took place during an observation period of six hours.

The salivary ducts are annulated tubes with thin chitinous walls 140 μ long and a lumen 7.5 μ wide (strikingly wider than the lumen of the salivary ducts of mosquitos which are 2.5 μ wide). They pass into the head and converge to the middle line where they unite to form the common salivary duct. The common salivary duct is 190 μ long and 11 μ wide and has the same structure as the salivary ducts; it passes in the middle of the head underneath the inferior ganglion of the brain and opens into the salivary pump. The inferior wall of the common salivary duct shortly before its entrance into the salivary pump joins the inferior lamina of the hypopharynx.

The salivary pump is elliptical, 100 μ long by 45 μ wide (Pl. X, fig. 2, and Text-fig. 3). Its walls are formed of thick chitin and are traversed interiorly by strongly-marked circular ridges. A little in front of the entrance of the common salivary duct the floor of the salivary pump contains a small yellow elevation from which a number of minute teeth project into the lumen of the pump. Anteriorly the lumen of the salivary pump is continuous with the salivary canal which pierces the hypopharynx.

We have to thank Mr. M. Ber, of Jerusalem, for collecting a large number of sandflies and for being the subject of numerous experiments.

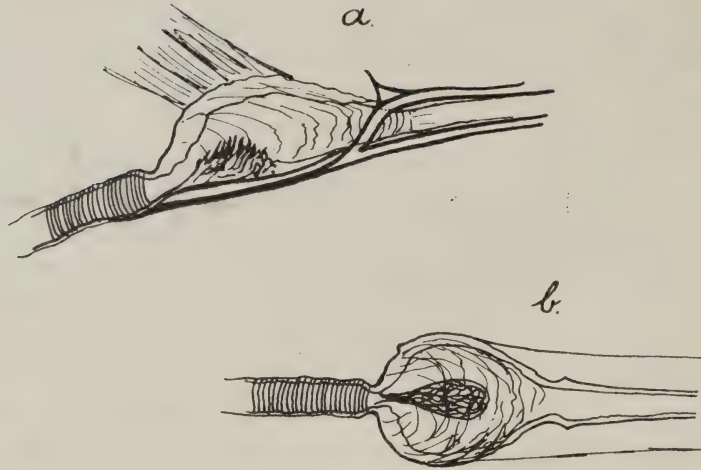


FIG. 3 (diagrammatic). Salivary pump. *a.*—side view; *b.*—dorsal view.

REFERENCES

- ADLER, S., and THEODOR, O. (1925). The Experimental Transmission of Cutaneous Leishmaniasis to Man from *Pblebotomus papatasi*. *Ann. Trop. Med. & Parasitol.*, Vol. XIX, No. 3, pp. 365-371.
- CHRISTOPHERS, S. R., SHORTT, H. E., and BARRAUD, P. J. (1925). Further Observations on the Feeding of Sandflies *Pblebotomus argentipes* on Cases of Kala-azar. *Ind. J. Med. Res.*, Vol. XIII, No. 1, pp. 159-165.
- GRASSI, B. (1907). Ricerche sui Flebotomi. *R. Accademia dei Lincei*. Rome.
- HOARE, C. A. (1921). An Experimental Study of the Sheep-Trypanosome (*T. melophagium* Flu, 1908) and its Transmission by the Sheep Ked (*Melophagus ovinus*). *Parasitology*, Vol. XV, No. 4, pp. 365-424.
- NEWSTEAD, R. (1911). The Papataci Flies of the Maltese Islands. *Ann. Trop. Med. & Parasitol.*, Vol. V, No. 2, pp. 139-186.
- PATTON, W. S., and CRAGG, W. F. (1913). A Textbook of Medical Entomology, pp. 29-35 and p. 109. Christian Literature Society for India. London, Madras, Calcutta.
- WATERSTON, J. (1922). A Contribution to the Knowledge of the Bionomics of Sandflies. *Ann. Trop. Med. & Parasitol.*, Vol. XVI, No. 1, pp. 69-92.

ADDENDUM

There are usually five prestomal teeth on each labellum, three set close together as described and figured above and two more distal than the others.

Since the above paper was written we have observed one instance of *P. papatasi* passing a minute amount of fluid per anum during the act of feeding but this is very exceptional and therefore not of aetiological importance.

(According to Parrot (1922) *P. minutus* var. *africanus* passes fluid per anum during feeding.)

Parrot (1922) also observed an interval between biting and the entrance of blood into the insect (temps préparatoire) ; the interval noted was three to six minutes in *P. minutus* var. *africanus* and a half to one minute in *P. papatasi*.

REFERENCE

- PARROT, L. (1922.) Recherches sur l'étiologie du Bouton d'Orient. *Bull. Soc. Path. Exot.*, Vol. XV, No. 1, pp. 80-92.

15 Jan., 1926

EXPLANATION OF PLATE VIII

Fig. 1. Sagittal section through the middle of the head of
P. papatasi. × 100.

b.m.—Muscles of the buccal cavity.

s.g.—Superior ganglion of the brain.

i.g.—Inferior ganglion of the brain.

c.s.d.—Common salivary duct.

s.m.—Salivary muscle inserted into salivary pump.

h.—Hypopharynx.

l.e.—Labrum-epipharynx.

Fig. 2. Sagittal section through pharynx and oesophageal valve.
× 130.

o.—Oesophagus.

o.d.—Oesophageal diverticulum.

o.v.—Oesophageal valve.

p.t.—Pharyngeal teeth.

Fig. 3. Connecting tube between pharynx and buccal cavity.
× 150.

c.t.—Connecting tube.

s.—Sphincter muscle.



FIG. 1



FIG. 2

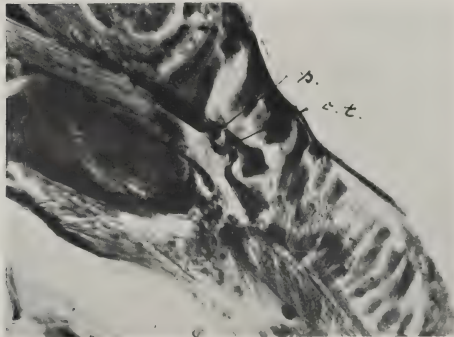


FIG. 3

THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS

PLATE IX

EXPLANATION OF PLATE IX

Transverse sections through the head and the proboscis.
(All figures $\times 300$.)

Fig. 1. Pharynx at its commencement.

Fig. 2. Pharynx showing teeth.

Fig. 3. Pharynx: the two lateral plates only are toothed.

Fig. 4. Pharynx immediately before entering the brain.

Fig. 5. Pharynx in the middle of the brain.

Fig. 6. Section through the clypeus.

s.p.—Salivary pump.

b.c.—Buccal cavity. Floor and side of the buccal cavity are thickly chitinised; the roof is thinly chitinised.

Figs. 7 and 8. Sections through the proboscis.

The sections are arranged progressively from behind forwards.

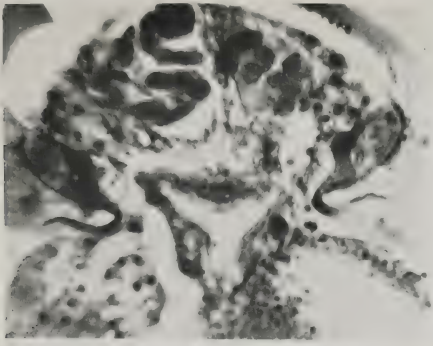


FIG. 1

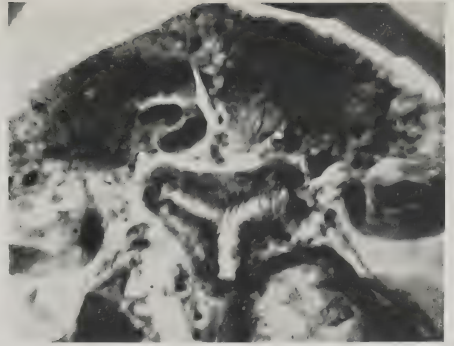


FIG. 2

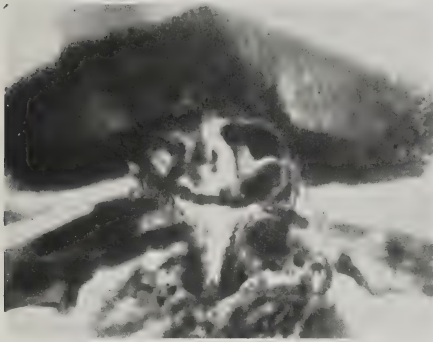


FIG. 3



FIG. 4

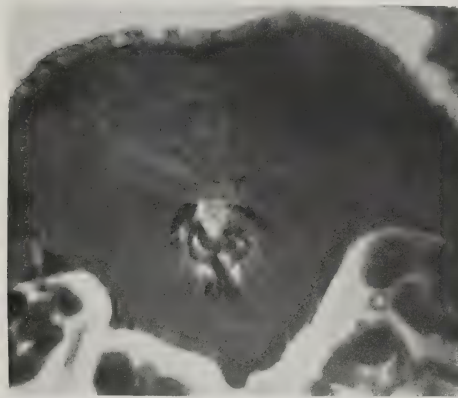


FIG. 5

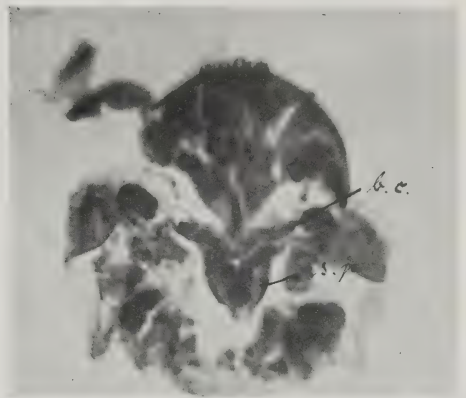


FIG. 6



FIG. 7

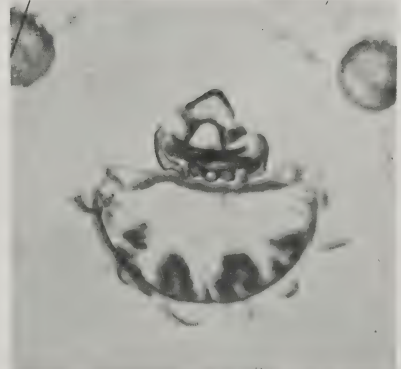


FIG. 8

THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS

PLATE X

EXPLANATION OF PLATE X

Fig. 1. Salivary glands.

Fig. 2. Lateral view of buccal cavity.

c.s.d.—Common salivary duct.

s.p.—Salivary pump.

el.—Elevation on the floor of the salivary pump from which minute teeth project.

Fig. 3. Dorsal view of the pharynx and the buccal cavity.

(All figures $\times 225$.)

b.c.—Buccal cavity.

p.—Pharynx.

i.b.—Chitinous arch beneath the buccal cavity from which the salivary muscle rises.



FIG. 1



FIG. 2



FIG. 3

THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

PLATE XI

EXPLANATION OF PLATE XI

Fig. 1. Mandible.

e.c.—External cornu.*ab* .—Abductor tendon of the mandible.*adc.*—Adductor tendon of the mandible.*i.c.*—Internal cornu.*r.*—Ridge into which the adductor tendon is inserted.

Fig. 2. Origin of the mandible.

sc.—Sclerite from which the mandible arises.*w.*—Wedge of chitin between the two cornua.

Fig. 3. Maxilla.

l.—Long process (intracranial).*b.*—Blade.*a.*—Anterior margin of the clypeus.*c.a.*—Chitinous arch at anterior part of the gular region from which the mentum arises.*pa.*—Palp.

Fig. 4. The head as seen in a cleared preparation from above.

i.c.t.—Intracranial tunnel.*ch.t.*—Chitinous tubes uniting the intracranial tunnels anteriorly.*p.*—Pharynx.*b.c.*—Buccal cavity.*i.b.*—Chitinous arch beneath the buccal cavity.*s.p.*—Salivary pump.*r.*—Ridge in front of the occipital foramen.*i.*—Invagination into the sides of the clypeus round which the abductor tendon of the mandible turns.*mn.*—Mandibles.*pa.*—Palp.